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# Synthesis, Characterization and Microbial Activity of N-Substituted Pyrazolines

JYOTI GABA[1,*], SUNITA SHARMA[2], GEETIKA ARORA[1] and POONAM SHARMA[2]

[1]Department of Chemistry, Punjab Agricultural University, Ludhiana-141 004, India
[2]Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana-141 004, India

*Corresponding author: E-mail: jyotgcw@gmail.com

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Acetic acid and propanoic acid were treated with thionyl chloride to give respective acid chlorides, which were then reacted with synthesized pyrazolines using triethylamine as catalyst in dry dichloromethane to give N-acetylated pyrazolines (**1b-7b**) and N-propanoylated pyrazolines (**1c-7c**). Synthesized N-substituted pyrazolines were characterized by IR, $^1$H NMR and $^{13}$C NMR spectral studies. Prepared compounds were screened for their microbial activity against *Bacillus* sp., *Pseudomonas* sp., *Acinetobactor* sp. and *Klebsiella* sp. N-acetylated (**7b**) and N-propanoylated (**7c**) pyrazolines having substitution of methoxy group at *meta* position and hydroxy group at *para* position of benzene ring were found effective against *Bacillus* sp., *Acinetobactor* sp. and *Pseudomonas* sp. but not against *Klebsiella* sp. All pyrazolines and N-substituted pyrazolines exhibited less activity than standard ampicillin at all the tested concentrations.

**Keywords: N-substituted pyrazolines, $^1$H NMR, $^{13}$C NMR, Microbial activity.**

## INTRODUCTION

Pyrazoles are well known important nitrogen containing 5-membered hetrocyclic compounds. The two nitrogen atoms are present in the adjacent position with two endocyclic double bonds. Pyrazoline is dihydropyrazole possessing only one endocyclic double bond. These electron rich nitrogen heterocycles play an important role in diverse biological activities. Several pyrazoline derivatives possess important pharmacological activities and therefore these are useful materials in drug research. Substituted pyrazolines are useful in pharmaceutical and agrochemical research. N-substituted pyrazoline derivatives also exhibit biological activities like anti-inflammatory, analgesic, antimicrobial, antitumor, antileukemia, antidepressant, angiotensin converting enzyme inhibitory activity and hypertension. Several applications of N-arylpyrazoles in medicines such as antitumor, antiviral, anti-inflammatory agents, kinase inhibitors for the treatment of type-2 diabetes, hyperlipidemia and obesity [1-4]. Keeping in view the potential biological activities of pyrazolines, it was perceived that the synergistic effect of heterocyclic moieties in single nucleus might result in the formation of some worth-while molecules from the biological point of view.

## EXPERIMENTAL

All the recorded melting points were determined in open capillaries and are uncorrected. Structures of compounds were confirmed by routine spectrometric analysis. IR, $^1$H and $^{13}$C NMR spectra were scanned from Sophisticated Analytical Instrumentation Facility (SAIF), Central Instrument Laboratory (CIL), Panjab University, Chandigarh. The IR spectra of compounds were recorded using KBr discs on Perkin-Elmer FTIR spectrophotometer. $^1$H NMR and $^{13}$C NMR spectra were recorded on a Bruker Avance II 400 MHz instrument using TMS as an internal standard.

**General procedure for synthesis of N-substituted pyrazolines (1b-7b, 1c-7c):** To a solution of acetic acid or propanoic acid (0.01 mol) in dry dichloromethane (50 mL) was slowly added thionyl chloride (0.01 mol) at 0 °C, then the solution was stirred at room temperature for 30 min and heated to 40 °C for 1 h. The resulting solution was stirred in ice-cold water. Already synthesized pyrazoline [5] (0.01 mol) was added to above reaction mixture in small lots. Triethylamine (0.01 mol) was added drop-wise from a dropping funnel over a period of 15 min. Then the reaction mixture was brought to room temperature and stirred for another 2 h. The solution was partitioned between dichloromethane and 2.7 N HCl (50 mL) and the two layers were separated. The aqueous layer was again extracted with dichloromethane (50 mL). Saturated aqueous sodium bicarbonate solution was added and the resulting mixture was transferred to a 500 mL separatory funnel. Layers were separated and aqueous phase was again extracted with dichloromethane (50 mL). The combined organic layers were dried over sodium sulphate (anhydrous)

and concentrated on rotary vacuum evaporator. The crude product was recrystallized from methanol to yield different N-substituted pyrazolines (**1b-7b, 1c-7c**).

**Testing of microbial activity:** The effect of N-substituted pyrazolines on the growth of *Bacillus* sp., *Pseudomonas* sp., *Acinetobacter* sp. and *Klebsiella* sp. was assessed by bacterial sensitivity-filter paper disc method. The plates were prepared by pouring 15-20 mL of the sterilized nutrient agar media (*Bacillus* sp.*, Acinetobacter* sp. and *Klebsiella* sp.) and King's B (*Pseudomonas* sp.) on sterilized petriplates. Plates were then allowed to solidify and stored for 2 days to ensure sterility. Suspension of 3-4 days old broth of the test organism was then spread on the required medium plates. Sterile filter paper discs moistened with test compound solution in dimethyl sulphoxide were carefully placed on the medium under aseptic conditions inoculated with the respective bacterial suspension. Sterilized filter paper discs dipped in dimethyl sulphoxide served as control. Plates were incubated at 28 ± 1 °C and the diameter of growth inhibition zone (mm) was measured after 24 h. The growth of the organism on medium containing the test compound was also compared with the growth on the plates containing micro-organism without test compound as control.

**1-(3-Methyl-5-phenyl-4,5-dihydro-1*H*-pyrazol-1-yl)ethanone (1b):** Yield 60 %, m.p.: 156-158 °C. IR (KBr, $\nu_{max}$, $cm^{-1}$): 3038 (aromatic C-H str.), 2928 $\nu_{as}(CH_2)$, 1631 (C=O str.), 1593 (C=N str.), 1540 (N-N str.), 1458 (C=C str.), 1363 (C-N str.), 868 (C-N bending) and 834 (C-H bending). $^1$H NMR ($CDCl_3$) δ (ppm): 2.06 (s, 3H, $CH_3$), 3.17 (s, 3H, $CH_3$), 2.55-2.62 (dd, 1H, $CH_2$) (*J* = 18.1, 4.2 Hz), 3.33-3.40 (dd, 1H, $CH_2$) (*J* = 18.5, 11.2 Hz), 5.25-5.29 (dd, 1H,CH) (*J* = 11.7, 4.6 Hz), 7.10-7.29 (m, 5H, Ar-H). $^{13}$C NMR ($CDCl_3$) δ (ppm): 20.21 ($CH_3$), 23.46 ($CH_3$-C=O), 47.22 ($CH_2$), 52.19 (CH), 158.93 (C=N), 170.32 (C=O) 118.39-149.43 (aromatic carbons).

**1-[5-(2-Chlorophenyl)-3-methyl-4,5-dihydro-1*H*-pyrazol-1-yl]ethanone (2b):** Yield 45 %, m.p.: 137-139 °C. IR (KBr, $\nu_{max}$, $cm^{-1}$): 3054 (aromatic C-H str.), 2918 $\nu_{as}(CH_2)$, 1630 (C=O str.), 1592 (C=N str.), 1529 (N-N str.), 1470 (C=C str.), 1373 (C-N str.), 1044 (C-Cl str.), 871 (C-N bending) and 833 (C-H bending). $^1$H NMR ($CDCl_3$) δ (ppm): 2.04 (s, 3H, $CH_3$), 3.12 (s, 3H, $CH_3$), 2.52-2.59 (dd, 1H, $CH_2$) (*J* = 18.4, 4.4), 3.35-3.41 (dd, 1H, $CH_2$) (*J* = 18.6, 11.3 Hz), 5.26-5.30 (dd, 1H, CH) (*J* = 11.4, 4.3 Hz), 7.44-7.69 (m, 4H, Ar-H). $^{13}$C NMR ($CDCl_3$) δ (ppm): 16.36 ($CH_3$), 24.41 ($CH_3$-C=O), 42.18 ($CH_2$), 60.13 (CH), 157.92 (C=N), 168.55 (C=O), 112.68-143.29 (aromatic carbons).

**1-[5-(4-Chlorophenyl)-3-methyl-4,5-dihydro-1*H*-pyrazol-1-yl]ethanone (3b):** Yield 60 %, m.p.: 152-154 °C. IR (KBr, $\nu_{max}$, $cm^{-1}$): 3049 (aromatic C-H str.), 2919 $\nu_{as}(CH_2)$, 1630 (C=O str.), 1591 (C=N str.), 1526 (N-N str.), 1468 (C=C str.), 1379 (C-N str.), 1041 (C-Cl str.), 863 (C-N bending) and 839 (C-H bending). $^1$H NMR ($CDCl_3$) δ (ppm): 2.01(s, 3H, $CH_3$), 3.19 (s, 3H, $CH_3$), 2.57-2.66 (dd, 1H, $CH_2$) (*J* = 18.0, 4.4), 3.28-3.36 (dd, 1H, $CH_2$) (*J* = 18.5, 11.5 Hz), 5.19-5.26 (dd, 1H, CH) (*J* = 11.7, 4.3 Hz), 7.41-7.64 (m, 4H, Ar-H). $^{13}$C NMR ($CDCl_3$) δ (ppm): 18.32 ($CH_3$), 24.81 ($CH_3$-C=O), 45.98 ($CH_2$), 54.89 (CH), 152.61 (C=N), 169.74 (C=O), 113.79-143.44 (aromatic carbons).

**1-[5-(2-Methoxyphenyl)-3-methyl-4,5-dihydro-1*H*-pyrazol-1-yl]ethanone (4b):** Yield 62 %, m.p.: 135-137 °C. IR (KBr, $\nu_{max}$, $cm^{-1}$): 2991 (aromatic C-H str.), 2933 $\nu_{as}(CH_2)$, 1627 (C=O str.), 1597 (C=N str.), 1531 (N-N str.), 1457 (C=C str.), 1369 (C-N str.), 1152 ($\nu_{as}$C-O str.), 1046 ($\nu_s$C-O str.), 870 (C-N bending) and 833 (C-H bending). $^1$H NMR ($CDCl_3$) δ (ppm): 2.09(s, 3H, $CH_3$), 3.10(s, 3H, $CH_3$), 3.75 (s, 3H, $CH_3$), 2.59-2.65 (dd, 1H, $CH_2$) (*J* = 18.2, 4.6), 3.29-3.36 (dd, 1H, $CH_2$) (*J* = 18.7, 11.2 Hz), 5.21-5.29 (dd, 1H, CH) (*J* = 11.7, 4.5 Hz), 7.39-7.56 (m, 4H, Ar-H). $^{13}$C NMR ($CDCl_3$) δ (ppm): 16.96 ($CH_3$), 20.63 ($CH_3$-C=O), 45.19 ($CH_2$), 56.71 (CH), 158.16 (C=N), 53.32 ($OCH_3$), 167.86 (C=O), 113.79-143.44 (aromatic carbons).

**1-[5-(3-Methoxyphenyl)-3-methyl-4,5-dihydro-1*H*-pyrazol-1-yl]ethanone (5b):** Yield 70 %, m.p.: 146-147 °C. IR (KBr, $\nu_{max}$, $cm^{-1}$): 2995 (aromatic C-H str.), 2930 $\nu_{as}(CH_2)$, 1633 (C=O str.), 1606 (C=N str.), 1528 (N-N str.), 1449 (C=C str.), 1370 (C-N str.), 1157 $\nu_{as}$(C-O str.), 1040 $\nu_s$(C-O str.), 868 (C-N bending) and 836 (C-H bending). $^1$H NMR ($CDCl_3$) δ (ppm): 2.08(s, 3H, $CH_3$), 3.12(s, 3H, $CH_3$), 3.79 (s, 3H, $CH_3$), 2.53-2.60 (dd, 1H, $CH_2$) (*J* = 18.4, 4.1), 3.36-3.44 (dd, 1H, $CH_2$) (*J* = 18.5, 11.4 Hz), 5.21-5.27 (dd, 1H, CH) (*J* = 11.8, 4.5Hz), 7.37-7.62 (m, 4H, Ar-H). $^{13}$C NMR ($CDCl_3$) δ (ppm): 20.12 ($CH_3$), 24.44 ($CH_3$-C=O), 42.81 ($CH_2$), 55.66 (CH), 153.89 (C=N), 52.81 ($OCH_3$), 169.10 (C=O), 108.39-147.66 (aromatic carbons).

**1-[5-(4-Hydroxyphenyl)-3-methyl-4,5-dihydro-1*H*-pyrazol-1-yl]ethanone (6b):** Yield 51 %, m.p.: 168-170. IR (KBr, $\nu_{max}$, $cm^{-1}$): 3432 (O-H str.), 3069 (aromatic C-H str.), 2970 $\nu_{as}(CH_2)$, 1632 (C=O str.), 1603 (C=N str.), 1517 (N-N str.), 1439 (C=C str.), 1352 (C-N str.), 1176 $\nu_{as}$(C-O str.), 1019 $\nu_s$(C-O str.), 847 (C-N bending) and 830 (C-H bending). $^1$H NMR (DMSO-*d*$_6$) δ (ppm): 2.09 (s, 3H, $CH_3$), 3.20 (s, 3H, $CH_3$), 2.53-2.60 (dd, 1H, $CH_2$) (*J* = 18.1, 4.5), 3.36-3.342 (dd, 1H, $CH_2$) (*J* = 18.7, 11.2 Hz), 5.23-5.27 (dd, 1H, CH) (*J* = 11.8, 4.6 Hz), 7.49-7.82 (m, 4H, Ar-H), 8.76 (s, 1H, OH). $^{13}$C NMR (DMSO-*d*$_6$) δ (ppm): 17.84 ($CH_3$), 21.46 ($CH_3$-C=O), 46.71 ($CH_2$), 57.11 (CH), 158.63 (C=N), 170.12 (C=O), 108.63-145.81 (aromatic carbons).

**1-[5-(4-Hydroxyphenyl)-3-methyl-4,5-dihydro-1*H*-pyrazol-1-yl]propan-1-one (7b):** Yield 76 %, m.p.: 125-128. IR (KBr, $\nu_{max}$, $cm^{-1}$): 3322 (O-H str.), 2955 (aromatic C-H str.), 2931 $\nu_{as}(CH_2)$, 1626 (C=O str.), 1590 (C=N str.), 1537 (N-N str.), 1460 (C=C str.), 1368 (C-N str.), 1133 $\nu_{as}$(C-O str.), 1032 $\nu_s$(C-O str.), 872 (C-N bending) and 837 (C-H bending). $^1$H NMR (DMSO-*d*$_6$) δ (ppm): 2.00 (s, 3H, $CH_3$), 3.16 (s, 3H, $CH_3$), 3.75 (s, 3H, $CH_3$), 2.58-2.64 (dd, 1H, $CH_2$) (*J* = 18.2, 4.3), 3.32-3.40 (dd, 1H, $CH_2$) (*J* = 18.5, 11.4 Hz), 5.22-5.26 (dd, 1H, CH) (*J* = 11.6, 4.4 Hz), 6.50-6.70 (m, 3H, Ar-H), 8.77 (s, 1H, OH). $^{13}$C NMR (DMSO-*d*$_6$) δ (ppm): 15.58 ($CH_3$), 21.64 ($CH_3$-C=O), 45.98 ($CH_2$), 58.61 (CH), 155.71 (C=N), 55.43 ($OCH_3$), 166.68 (C=O), 109.48-147.36 (aromatic carbons).

**1-(3-Methyl-5-phenyl-4,5-dihydro-1*H*-pyrazol-1-yl)propan-1-one (1c):** Yield 66 %, m.p.:178-180 °C. IR (KBr, $\nu_{max}$, $cm^{-1}$): 3042 (aromatic C-H str.), 2915 $\nu_{as}(CH_2)$, 1634 (C=O str.), 1589 (C=N str.), 1533 (N-N str.), 1465 (C=C str.), 1371 (C-N str.), 870 (C-N bending) and 833 (C-H bending). $^1$H NMR

($CDCl_3$) δ (ppm): 2.07 (s, 3H, $CH_3$), 3.22 (q, 2H, $CH_2$), 2.20 (t, 3H, $CH_3$), 2.55-2.62 (dd, 1H, $CH_2$) (*J* = 18.2, 4.4), 3.28-3.36 (dd, 1H, $CH_2$) (*J* = 18.4, 11.6 Hz), 5.23-5.27 (dd, 1H, CH) (*J* = 11.56, 4.4 Hz), 7.19-7.44 (m 5H, Ar-H). $^{13}$C NMR ($CDCl_3$) δ (ppm): 19.43 ($CH_3$), 23.98 (**C**$H_3$-$CH_2$-C=O), 35.53 (**C**$H_2$-C=O), 45.18 ($CH_2$), 57.77 (CH), 154.48 (C=N), 163.62 (C=O), 120.44-153.81 (aromatic carbons).

**1-[5-(2-Chlorophenyl)-3-methyl-4,5-dihydro-1*H*-pyrazol-1-yl]propan-1-one (2c):** Yield 55 %, m.p.: 149-151 °C. IR (KBr, $\nu_{max}$, $cm^{-1}$): 3049 (aromatic C-H str.), 2922 $\nu_{as}(CH_2)$, 1628 (C=O str.), 1598 (C=N str.), 1540 (N-N str.), 1467 (C=C str.), 1379 (C-N str.), 1030 (C-Cl str.), 874 (C-N bending) and 835 (C-H bending). $^1$H NMR ($CDCl_3$) δ (ppm): 2.10 (s, 3H, $CH_3$), 3.26 (q, 2H $CH_2$), 2.16 (t, 3H, $CH_3$), 2.49-2.56 (dd, 1H, $CH_2$) (*J* = 18.1, 4.5), 3.38-3.45 (dd, 1H, $CH_2$) (*J* = 18.3, 11.4 Hz), 5.25-5.32 (dd, 1H, CH) (*J* = 11.8, 4.6 Hz), 7.46-7.59 (m 4H, Ar-H). $^{13}$C NMR ($CDCl_3$) δ (ppm): 17.32 ($CH_3$), 21.12 (**C**$H_3$-$CH_2$-C=O), 34.69 (**C**$H_2$-C=O), 48.42 ($CH_2$), 61.87 (CH), 152.13 (C=N), 163.56 (C=O), 109.48- 145.19 (aromatic carbons).

**1-[5-(4-Chlorophenyl)-3-methyl-4,5-dihydro-1*H*-pyrazol-1-yl]propan-1-one (3c):** Yield 62 %, m.p.: 160-162 °C. IR (KBr, $\nu_{max}$, $cm^{-1}$): 3044 (aromatic C-H str.), 2917 $\nu_{as}(CH_2)$, 1634 (C=O str.), 1596 (C=N str.), 1530 (N-N str.), 1464 (C=C str.), 1383 (C-N str.), 1032 (C-Cl str.), 862 (C-N bending) and 836 (C-H bending). $^1$H NMR ($CDCl_3$) δ (ppm): 2.02 (s, 3H, $CH_3$), 3.22 (q, 2H $CH_2$), 2.14 (t, 3H, $CH_3$), 2.56-2.65 (dd, 1H, $CH_2$) (*J* = 18.5, 4.4), 3.36-3.42 (dd, 1H, $CH_2$) (*J* = 18.5, 11.3 Hz), 5.27-5.33 (dd, 1H, CH) (*J* = 11.6, 4.6 Hz), 7.38-7.54 (m, 4H, Ar-H). $^{13}$C NMR ($CDCl_3$) δ (ppm): 17.85 ($CH_3$), 22.56 (**C**$H_3$-$CH_2$-C=O), 31.82 (**C**$H_2$-C=O), 48.79 ($CH_2$), 56.16 (CH), 153.87 (C=N), 162.59 (C=O), 114.76-152.83 (aromatic carbons).

**1-[5-(2-Methoxyphenyl)-3-methyl-4,5-dihydro-1*H*-pyrazol-1-yl]propan-1-one (4c):** Yield 65 %, m.p.: 150-152 °C. IR (KBr, $\nu_{max}$, $cm^{-1}$): 2985 (aromatic C-H str.), 2923 $\nu_{as}(CH_2)$, 1630 (C=O str.), 1592 (C=N str.), 1527 (N-N str.), 1453 (C=C str.), 1372 (C-N str.), 1144 $\nu_{as}$(C-O str.), 1050 $\nu_s$(C-O str.), 870 (C-N bending) and 825 (C-H bending). $^1$H NMR ($CDCl_3$) δ (ppm): 2.02 (s, 3H, $CH_3$), 3.78 (s, 3H, $CH_3$), 3.25 (q, 2H, $CH_2$), 2.19 (t, 3H, $CH_3$), 2.52-2.58 (dd, 1H, $CH_2$) (*J* = 18.1, 4.5), 3.34-3.40 (dd, 1H, $CH_2$) (*J* = 18.5, 11.2 Hz), 5.22-5.26 (dd, 1H, CH) (*J* = 11.4, 4.6 Hz), 7.44-7.66 (m, 4H, Ar-H). $^{13}$C NMR ($CDCl_3$) δ (ppm): 14.82 ($CH_3$), 23.81 (**C**$H_3$-$CH_2$-C=O), 29.89 (**C**$H_2$-C=O), 45.59 ($CH_2$), 56.18 (CH), 158.75 (C=N), 54.53 ($OCH_3$), 165.66 (C=O), 107.38-149.54 (aromatic carbons).

**1-[5-(3-Methoxyphenyl)-3-methyl-4,5-dihydro-1*H*-pyrazol-1-yl]propan-1-one (5c):** Yield 69 %, m.p.: 151-153 °C. IR (KBr, $\nu_{max}$, $cm^{-1}$): 2988 (aromatic C-H str.), 2923 $\nu_{as}(CH_2)$, 1638 (C=O str.), 1610 (C=N str.), 1531 (N-N str.),1451 (C=C str.), 1377 (C-N str.), 1150 $\nu_{as}$(C-O str.), 1049 $\nu_s$(C-O str.), 863 (C-N bending) and 830 (C-H bending). $^1$H NMR ($CDCl_3$) δ (ppm): 2.00 (s, 3H, $CH_3$), 3.81 (s, 3H, $CH_3$), 3.15 (q, 2H $CH_2$), 2.17 (t, 3H, $CH_3$), 2.52-2.62 (dd, 1H, $CH_2$) (*J* = 18.3, 4.6), 3.39-3.47 (dd, 1H, $CH_2$) (*J* = 18.6, 11.1 Hz), 5.22-5.35 (dd, 1H, CH) (*J* = 11.3, 4.2 Hz), 7.40-7.66 (m, 4H, Ar-H). $^{13}$C NMR ($CDCl_3$) δ (ppm): 14.83 ($CH_3$), 24.66 (**C**$H_3$-$CH_2$-C=O), 29.81 (**C**$H_2$-C=O), 44.61 ($CH_2$), 58.73 (CH), 155.97 (C=N), 54.42 ($OCH_3$), 166.65 (C=O), 110.36-146.87 (aromatic carbons).

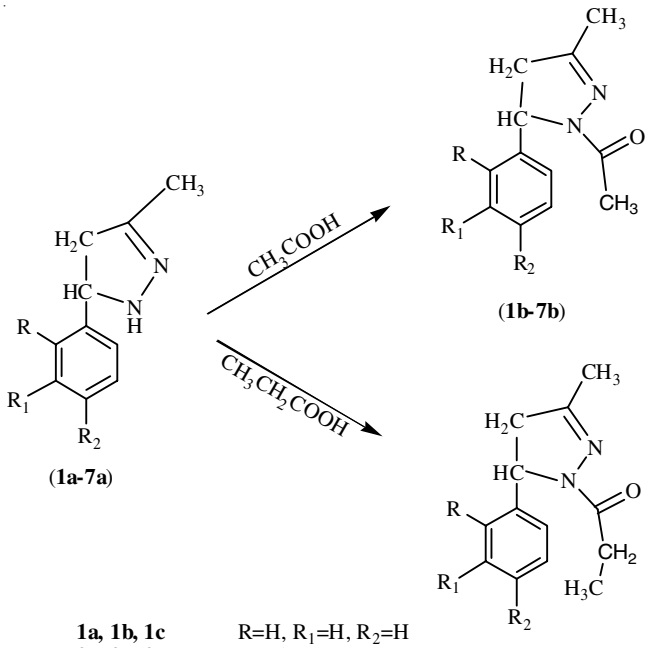
**1-[5-(4-Hydroxyphenyl)-3-methyl-4,5-dihydro-1*H*-pyrazol-1-yl]propan-1-one (6c):** Yield 73 %, m.p.: 189-190 °C. IR (KBr, $\nu_{max}$, $cm^{-1}$): 3429 (O-H str.), 3072 (aromatic C-H str.), 2968 $\nu_{as}(CH_2)$, 1635 (C=O str.), 1608 (C=N str.), 1520 (N-N str.), 1445 (C=C str.), 1358 (C-N str.), 1166 $\nu_{as}$(C-O str.), 1029 $\nu_s$(C-O str.), 843 (C-N bending) and 835 (C-H bending). $^1$H NMR (DMSO-$d_6$) δ (ppm): 2.05 (s, 3H, $CH_3$), 3.25 (q, 2H $CH_2$), 2.26 (t, 3H, $CH_3$), 2.54-2.62 (dd, 1H, $CH_2$) (*J* = 18.1, 4.4), 3.32-3.41 (dd, 1H, $CH_2$) (*J* = 18.3, 11.3 Hz), 5.25-5.37 (dd, 1H, CH) (*J* = 11.7, 4.8 Hz), 7.52-7.85 (m, 4H, Ar-H), 8.80 (s, 1H, OH). $^{13}$C NMR (DMSO-$d_6$) δ (ppm): 18.88 ($CH_3$), 21.68 (**C**$H_3$-$CH_2$-C=O), 31.65 (**C**$H_2$-C=O), 44.16 ($CH_2$), 57.79 (CH), 158.81 (C=N), 167.38 (C=O), 111.39-149.36 (aromatic carbons).

**1-[5-(4-Hydroxy-3-methoxyphenyl)-3-methyl-4,5-dihydro-1*H*-pyrazol-1-yl]propan-1-one (7c):** Yield 55 %, m.p.: 154-155 °C. IR (KBr, $\nu_{max}$, $cm^{-1}$): 3323 (O-H str.), 2961 (aromatic C-H str.), 2926 $\nu_{as}(CH_2)$, 1627 (C=O str.), 1599 (C=N str.), 1527 (N-N str.),1452 (C=C str.), 1374 (C-N str.), 1124 $\nu_{as}$(C-O str.), 1037 $\nu_s$(C-O str.), 868 (C-N bending) and 828 (C-H bending). $^1$H NMR (DMSO-$d_6$) δ (ppm): 2.00 (s, 3H, $CH_3$), 3.75 (s, 3H, $CH_3$), 3.35 (q, 2H $CH_2$), 2.16 (t, 3H, $CH_3$), 2.51-2.64 (dd, 1H, $CH_2$) (*J* = 18.3, 4.4), 3.32-3.40 (dd, 1H, $CH_2$) (*J* = 18.5, 11.4 Hz), 5.22-5.26 (dd, 1H, CH) (*J* = 11.5, 4.4 Hz), 6.49-6.70 (m, 4H, Ar-H), 8.79 (s, 1H, OH). $^{13}$C NMR (DMSO-$d_6$) δ (ppm): 15.58 ($CH_3$), 21.65 (**C**$H_3$-$CH_2$-C=O), 54.45 (**C**$H_2$-C=O), 45.97 ($CH_2$), 58.61 (CH), 155.78 (C=N), 55.44 ($OCH_3$), 166.65 (C=O), 109.54-147.38 (aromatic carbons).

## RESULTS AND DISCUSSION

Desired N-substituted pyrazolines were synthesized according to steps depicted in Fig. 1. Pyrazolines are weak bases, which can react with acids to form salts. Acids were first changed to acid chlorides by reaction with thionyl chloride. Addition of amine (pyrazoline) (**1b-7b**) in small lots to acid chloride solution in dry dichloromethane in presence of triethylamine was done to neutralize one equivalent of acid formed. So triethylamine acted as a catalyst in preparation of N-substituted pyrazolines (**1b-7b** and **1c-7c**). As thionyl chloride reacts explosively with water, use of dry solvent is necessary. The physical parameters (colour, yield, melting point, $R_f$ values) of synthesized compounds were determined and are presented in Table-1. All compounds were characterized by their spectral data (IR, $^1$H NMR and $^{13}$C NMR).

**IR data:** Infrared spectral data revealed the formation of N-substituted pyrazolines. N-N, C=N and C-N stretching were also observed in range of 1527-1514, 1606-1590 and 1378-1348 $cm^{-1}$, respectively which assured the formation of five membered pyrazoline ring. The presence of C=C in aromatic ring was confirmed due to presence of band in 1474-1431 $cm^{-1}$, region. Band for O-H stretching was observed for compounds **6b, 7b, 6c** and **7c** having hydroxy group on aromatic ring.




| | |
|---|---|
| **1a, 1b, 1c** | R=H, $R_1$=H, $R_2$=H |
| **2a, 2b, 2c** | R=Cl, $R_1$=H, $R_2$=H |
| **3a, 3b, 3c** | R=H, $R_1$=H, $R_2$=Cl |
| **4a, 4b, 4c** | R=$OCH_3$, $R_1$=H, $R_2$=H |
| **5a, 5b, 5c** | R=H, $R_1$=$OCH_3$, $R_2$=H |
| **6a, 6b, 6c** | R=H, $R_1$=H, $R_2$=OH |
| **7a, 7b, 7b** | R=H, $R_1$=$OCH_3$, $R_2$=OH |

Fig. 1. Scheme for the synthesis of N-substituted pyrazolines

TABLE-1
PHYSICAL PARAMETERS OF N-SUBSTITUTED PYRAZOLINES

| Compd. | m.f. | Colour | Yield (%) | m.p. (°C) | $R_f$ |
|---|---|---|---|---|---|
| **1b** | $C_{12}H_{14}N_2O$ | Brown | 60 | 156-158 | 0.54 |
| **1c** | $C_{13}H_{16}N_2O$ | Brown | 66 | 178-180 | 0.54 |
| **2b** | $C_{12}H_{13}N_2OCl$ | Brown | 45 | 137-139 | 0.53 |
| **2c** | $C_{13}H_{15}N_2OCl$ | Brown | 55 | 149-151 | 0.52 |
| **3b** | $C_{12}H_{13}N_2OCl$ | Brown | 60 | 152-154 | 0.52 |
| **3c** | $C_{13}H_{15}N_2OCl$ | Brown | 62 | 160-162 | 0.52 |
| **4b** | $C_{13}H_{16}N_2O_2$ | Brown | 62 | 135-137 | 0.53 |
| **4c** | $C_{14}H_{18}N_2O_2$ | Dark brown | 65 | 150-152 | 0.52 |
| **5b** | $C_{13}H_{16}N_2O_2$ | Light brown | 70 | 146-147 | 0.53 |
| **5c** | $C_{14}H_{18}N_2O_2$ | Brown | 69 | 151-153 | 0.52 |
| **6b** | $C_{12}H_{14}N_2O_2$ | Yellow | 51 | 168-170 | 0.51 |
| **6c** | $C_{13}H_{16}N_2O_2$ | Yellow | 73 | 189-190 | 0.52 |
| **7b** | $C_{13}H_{16}N_2O_3$ | Reddish brown | 76 | 125-127 | 0.51 |
| **7c** | $C_{14}H_{18}N_2O_3$ | Off-white | 55 | 154-155 | 0.51 |

**$^1$H NMR data:** The formation of desired N-substituted pyrazolines was confirmed by $^1$H NMR spectrum. The two hydrogens on C-4 of pyrazoline ring (Fig. 2) were not found equivalent. Double doublets were obtained for both of these protons at 2.51-2.65 ppm and 3.22-3.41 ppm. Proton at C-5 of pyrazoline also appeared as double doublet in the range of 5.19-5.17 ppm. Also a singlet due to methyl group attached to carbonyl carbon appeared in spectra of N-acetylated pyrazolines. Similarly spectra of N-propanoylated pyrazolines exhibited a triplet of three methyl protons and a quartet of two methylene carbons attached to carbonyl carbon. Aromatic protons appeared as multiplet in range of 6.50-7.71 ppm. More confirmation to the formation of N-substituted pyrazolines was provided by coupling constant values of double doublets.

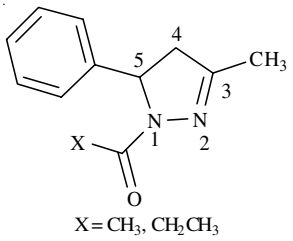



Fig. 2. Structure of pyrazoline ring

**$^{13}$C NMR data:** A further more assurance to the formation of desired compounds was added by $^{13}$C NMR data. Out of three carbons of pyrazoline ring, C-3 of pyrazoline ring (Fig. 2) in contact with electronegative nitrogen atom corresponding to C=N showed a maximum downfield shift. C-4 and C-5 of pyrazoline ring appeared near 45.59 ppm and 51.13 ppm respectively. Maximum absorption was observed for carbonyl carbon in the range of 162.59-170.32 ppm. The $^{13}$C NMR spectra of N-acetylated pyrazolines exhibited one peak in range of 20.62-24.44 ppm due to methyl carbon attached to carbonyl carbon. Similarly spectra of N-propanoylated pyrazoline exhibited peaks at 28.89-35.53 ppm and 21.12-24.66 ppm due to methylene and methyl carbon bonded to carbonyl group. The $^{13}$C NMR spectra of compounds **4b, 5b, 7b, 4c, 5c** and **7c** showed peak at 52.81-57.34 ppm indicating the presence of methoxy ($OCH_3$) group on benzene ring.

## Microbial activity

***Bacillus* sp.:** Table-2 showed that all the compounds except **2c**, **3c** and **4b** were active against *Bacillus* sp. at 250 µg/mL. N-propanoylated pyrazoline having methoxy group at *meta* position of benzene ring (**4c**) was significantly higher active than other compounds with an inhibition zone of 11 mm at 250 µg/mL.

TABLE-2
MICROBIAL ACTIVITY OF DIFFERENT N-SUBSTITUTED PYRAZOLINES ON THE GROWTH OF *Bacillus* sp.

| Compd. | Diameter of growth inhibition zone (mm) | | | | |
|---|---|---|---|---|---|
| | 3000 µg/mL | 2000 µg/mL | 1000 µg/mL | 500 µg/mL | 250 µg/mL |
| **1b** | 11.5 | 11.5 | 11.0 | 10.5 | 10.0 |
| **1c** | 12.0 | 11.5 | 11.0 | 10.5 | 9.5 |
| **2b** | 10.5 | 10.5 | 10.0 | 10.0 | 8.5 |
| **2c** | 11.5 | 11.0 | 10.0 | 9.5 | 0 |
| **3b** | 9.5 | 9.5 | 9.5 | 8.5 | 8.5 |
| **3c** | 11.5 | 11.0 | 10.0 | 9.0 | 0 |
| **4b** | 11.5 | 10.5 | 10.0 | 8.0 | 0 |
| **4c** | 11.5 | 10.5 | 9.0 | 8.5 | 8.5 |
| **5b** | 11.0 | 11.0 | 10.5 | 10.0 | 10.0 |
| **5c** | 12.0 | 12.0 | 12.0 | 11.0 | 11.0 |
| **6b** | 12.0 | 11.5 | 11.0 | 10.5 | 9.5 |
| **6c** | 12.0 | 11.5 | 11.5 | 10.0 | 10.0 |
| **7b** | 12.0 | 11.5 | 11.5 | 10.0 | 9.5 |
| **7c** | 12.0 | 11.0 | 10.5 | 10.5 | 9.5 |
| Ampicillin | 28.0 | 27.0 | 18.5 | 17.5 | 15.5 |
| CD (p = 0.05) | 0.22 | 0.44 | 0.44 | 0.17 | 0.44 |

Minimum inhibitory concentration (MIC) studies of N-acetylated pyrazolines (Fig. 3) revealed that compound **5b** having methoxy group at *meta* position of benzene ring was active against *Bacillus* sp. at concentration 190 µg/mL.

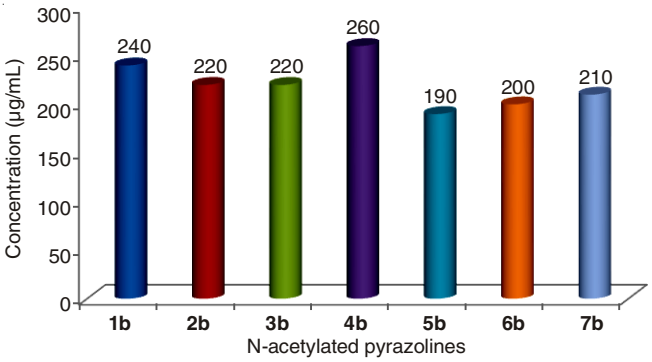



Fig. 3. Minimum inhibitory concentration (MIC) of different N-acetylated pyazolines against *Bacillus* sp.

N-propanoylated pyarzolines which were active against *Bacillus* sp. were further studied for minimum inhibitory concentration (MIC) values (Fig. 4). Compound **6c** having *para* hydroxy group on benzene ring was most active with minimum inhibitory concentration (MIC) value of 140 µg/mL. So it may be concluded that with the introduction of spacer, MIC value of all the compounds decreased except compounds **2c** and **3c** having chloro group at *ortho* and at *para* position respectively of benzene ring against *Bacillus* sp.

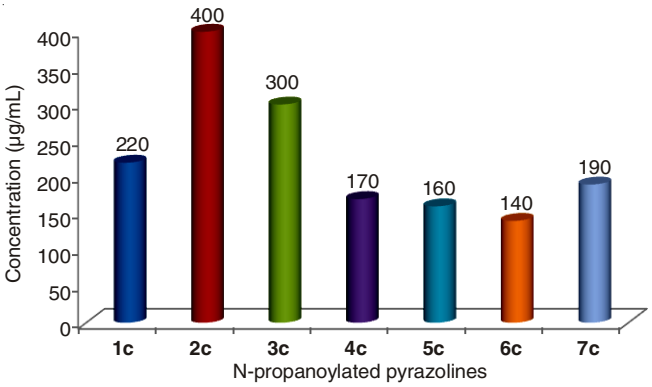



Fig. 4. Minimum inhibitory concentration (MIC) of different N-propanoylated pyazolines against *Bacillus* sp.

***Pseudomonas* sp.:** Table-3 revealed the inhibitory effect of different N-substituted pyrazolines on *Pseudomonas* sp. At 1000 µg/mL, compounds **1b**, **2b**, **4c** and **6b** exhibited higher activity as compared to other compounds with inhibition zone in range of 11.5-13.0 mm. N-propanoylated pyrazoline with methoxy group at *ortho* position of benzene ring (compound **4c**) showed maximum inhibition zones of 15, 14.5 and 13.0 mm at 3000, 2000 and 1000 µg/mL, respectively.

Minimum inhibitory concentration (MIC) studies of N-acetylated pyrazolines (Fig. 5) revealed that compound **7b** (having both hydroxy and methoxy groups on benzene ring) exhibited lowest MIC value of 150 µg/mL. Minimum inhibitory concentration (MIC) studies of different N-propanoylated pyrazolines (Fig. 6) suggested that presence of *meta* methoxy group (compound **5c**), *para* hydroxy group (compound **6c**) and both methoxy and hydroxy groups (compound **7c**) lowered the MIC values to a great extent as compared to substituted N-propanoylated pyrazoline **1c**. So it may be concluded that both N-acetylated pyrazolines (**7b**) and N-propanoylated pyrazolines having *ortho* methoxy group and *para* hydroxy

TABLE-3
MICROBIAL ACTIVITY OF DIFFERENT N-SUBSTITUTED PYRAZOLINES ON THE GROWTH OF *Pseudomonas* sp.

| Compd. | Diameter of growth inhibition zone (mm) | | | | |
|---|---|---|---|---|---|
| | 3000 µg/mL | 2000 µg/mL | 1000 µg/mL | 500 µg/mL | 250 µg/mL |
| **1b** | 12.0 | 12.0 | 11.5 | 11.5 | 11.0 |
| **1c** | 14.5 | 130 | 10.5 | 9.5 | 0 |
| **2b** | 12.5 | 11.5 | 11.5 | 11.0 | 9.0 |
| **2c** | 10.5 | 10.5 | 9.5 | 8.5 | 0 |
| **3b** | 11.0 | 11.0 | 11.0 | 9.5 | 8.0 |
| **3c** | 12.0 | 9.5 | 8.5 | 8.5 | 0 |
| **4b** | 11.5 | 10.0 | 9.5 | 9.0 | 9.0 |
| **4c** | 15.0 | 14.5 | 13.0 | 11.0 | 0 |
| **5b** | 11.5 | 10.5 | 10.0 | 10.0 | 9.5 |
| **5c** | 13.5 | 11.0 | 11.0 | 10.0 | 9.5 |
| **6b** | 13.0 | 12.5 | 11.5 | 10.0 | 10.0 |
| **6c** | 13.0 | 12.0 | 11.0 | 11.0 | 10.5 |
| **7b** | 12.5 | 11.5 | 11.0 | 11.0 | 10.0 |
| **7c** | 12.0 | 11.0 | 11.0 | 10.0 | 9.5 |
| Ampicillin | 24.0 | 20.0 | 18.0 | 16.5 | 15.0 |
| CD (p = 0.05) | 0.44 | 0.22 | 0.17 | 0.17 | 0.44 |

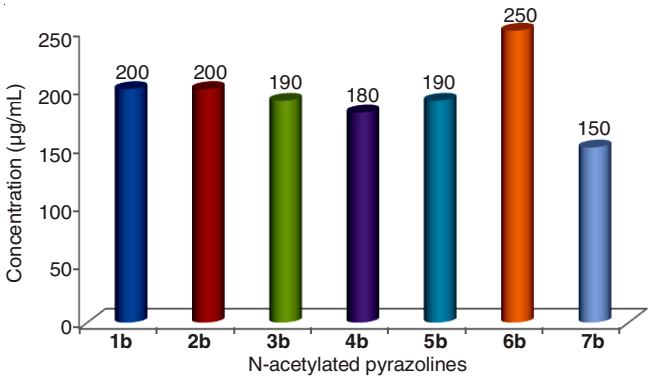



Fig. 5. Minimum inhibitory concentration (MIC) of different N-acetylated pyazolines against *Pseudomonas* sp.

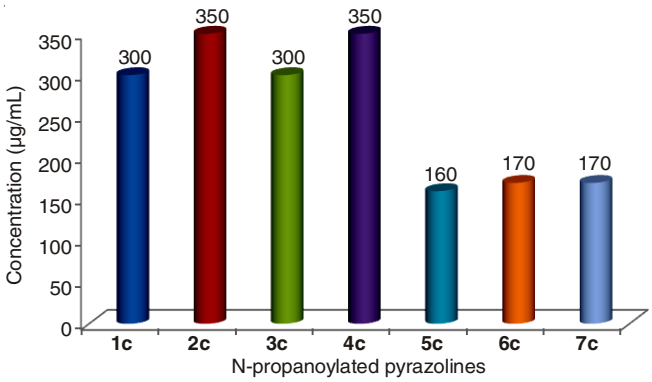



Fig. 6. Minimum inhibitory concentration (MIC) of different N-propanoylated pyazolines against *Pseudomonas* sp.

group (**7c**) exhibited considerably low MIC values (150 and 170 µg/mL) than unsubstituted pyrazolines (**1b** and **1c**). Compounds having electron donating groups like alkoxy and hydroxy proved to be beneficial and exhibited excellent antibacterial activity against *Pseudomonas* sp. [6,7].

***Acinetobacter* sp.:** Perusal of data given in Table-4 showed that N-propanoylated pyrazoline (**7c**) having both hydroxy and methoxy group on benzene ring exhibited highest activity against *Acinetobacter sp.* at all the concentrations. N-Acetylated

TABLE-4
MICROBIAL ACTIVITY OF DIFFERENT N-SUBSTITUTED PYRAZOLINES ON THE GROWTH OF *Acinetobacter* sp.

| Compd. | Diameter of growth inhibition zone (mm) | | | | |
|---|---|---|---|---|---|
| | 3000 µg/mL | 2000 µg/mL | 1000 µg/mL | 500 µg/mL | 250 µg/mL |
| **1b** | 14.0 | 13.0 | 12.5 | 12.5 | 11.0 |
| **1c** | 12.0 | 11.5 | 9.0 | 8.5 | 0 |
| **2b** | 14.5 | 11.5 | 11.0 | 9.5 | 0 |
| **2c** | 11.5 | 11.5 | 10.5 | 9.0 | 0 |
| **3b** | 11.5 | 11.0 | 11.0 | 10.0 | 9.0 |
| **3c** | 12.0 | 12.0 | 11.0 | 9.0 | 9.0 |
| **4b** | 12.0 | 11.5 | 10.0 | 10.0 | 9.0 |
| **4c** | 12.0 | 12.0 | 11.5 | 9.5 | 9.0 |
| **5b** | 12.0 | 11.0 | 11.0 | 10.0 | 10.0 |
| **5c** | 12.0 | 11.5 | 10.5 | 9.0 | 0 |
| **6b** | 12.0 | 12.0 | 11.0 | 11.0 | 0 |
| **6c** | 15.0 | 13.5 | 13.0 | 12.0 | 9.0 |
| **7b** | 14.0 | 12.0 | 11.0 | 10.0 | 9.0 |
| **7c** | 15.0 | 14.0 | 14.0 | 13.0 | 12.5 |
| Ampicillin | 25.0 | 23.0 | 17.0 | 16.5 | 15.0 |
| CD (p = 0.05) | 0.17 | 0.17 | 0.44 | 0.17 | 0.17 |

pyrazolines with no substitution on benzene ring (**1b**) and *ortho* chloro group (**2b**) were found more effective than respective N-propanoylated pyrazolines from 500 to 3000 µg/mL. Inhibition effect of standard was significantly higher than all tested compounds at all concentrations. So all N-acetylated pyrazolines exhibited lower MIC values as compared to all N-propanoylated pyrazolines except compound **4c** having chloro group at *para* position. Both N-acetylated pyrazolines and N-propanoylated pyrazolines having *para* chloro group registered lowest MIC values of 190 µg/mL and 120 µg/mL, respectively (Figs. 7 and 8).

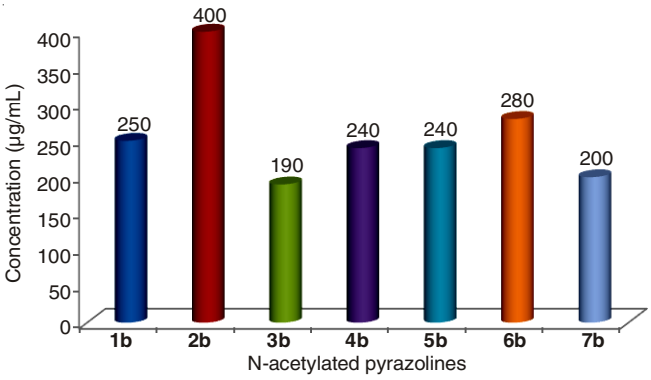



Fig. 7. Minimum inhibitory concentration (MIC) of different N-acetylated pyazolines against *Acinetobacter* sp.

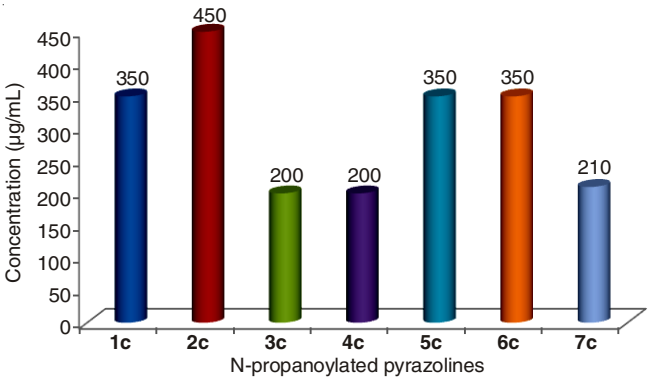



Fig. 8. Minimum inhibitory concentration (MIC) of different N-propanoylated pyazolines against *Acinetobacter* sp.

***Klebsiella* sp.:** Data presented in Table-5 exhibited the inhibitory effect of different N-substituted pyrazolines on *Klebsiella* sp. Compounds **3c** (*para* chloro group on benzene ring) and **6b** (*para* hydroxy group on benzene ring) exhibited highest inhibiton zones of 13.5 mm and 13.0 mm respectively at 250 µg/mL. Minimum inhibitory concentration values (MIC) of different N-acetylated pyrazolines against *Klebsiella* sp. are presented in Fig. 9. Data revealed that compound **1b** (no substitution on benzene ring) and **6b** (hydroxy group at *para* position of benzene ring) exhibited least MIC value of 150 µg/mL. Minimum inhibitory concentration (MIC) studies of different N-propanoylated pyrazolines (Fig. 10) exhibited that

TABLE-5
MICROBIAL ACTIVITY OF DIFFERENT N-SUBSTITUTED PYRAZOLINES ON THE GROWTH OF *Klebsiella* sp.

| Compd. | Diameter of growth inhibition zone (mm) | | | | |
|---|---|---|---|---|---|
| | 3000 µg/mL | 2000 µg/mL | 1000 µg/mL | 500 µg/mL | 250 µg/mL |
| **1b** | 13.5 | 13.5 | 12.0 | 11.5 | 11.0 |
| **1c** | 13.0 | 13.0 | 12.0 | 11.0 | 9.0 |
| **2b** | 11.5 | 10.5 | 10.5 | 10.0 | 9.5 |
| **2c** | 11.5 | 11.5 | 10.5 | 9.0 | 0 |
| **3b** | 13.0 | 12.0 | 11.5 | 10.0 | 9.0 |
| **3c** | 15.5 | 14.5 | 14.5 | 13.5 | 13.5 |
| **4b** | 14.5 | 14.0 | 13.5 | 13.5 | 12.5 |
| **4c** | 12.5 | 12.5 | 12.0 | 11.0 | 10.0 |
| **5b** | 12.0 | 12.0 | 11.5 | 11.0 | 10.0 |
| **5c** | 13.0 | 12.5 | 11.0 | 10.5 | 9.5 |
| **6b** | 14.5 | 14.0 | 13.5 | 13.5 | 13.0 |
| **6c** | 12.0 | 11.5 | 10.5 | 10.5 | 9.0 |
| **7b** | 12.0 | 12.0 | 11.5 | 11.0 | 11.0 |
| **7c** | 12.0 | 11.5 | 11.0 | 10.5 | 10.5 |
| Ampicillin | 30.0 | 23.0 | 17.0 | 16.5 | 15.0 |
| CD (p = 0.05) | 0.17 | 0.17 | 0.17 | 0.17 | 0.17 |

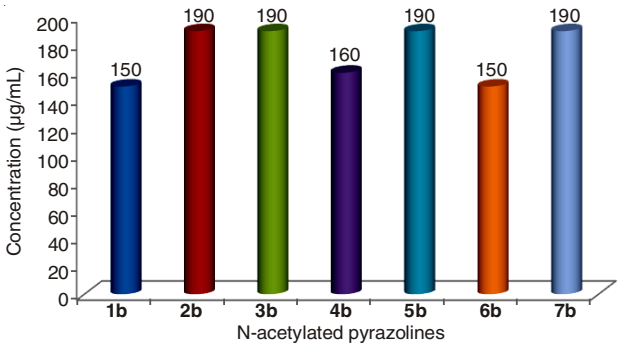



Fig. 9. Minimum inhibitory concentration (MIC) of different N-acetylated pyazolines against *Klebsiella* sp.

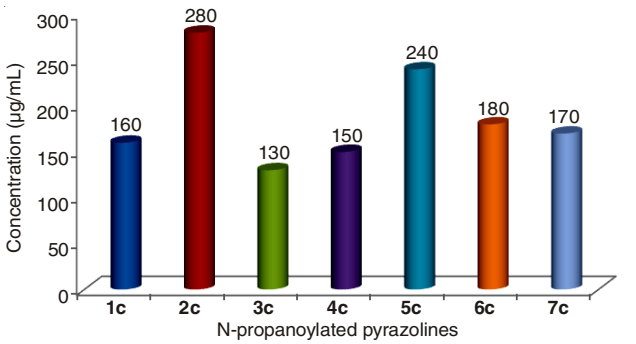



Fig. 10. Minimum inhibitory concentration (MIC) of different N-propanoylated pyazolines against *Klebsiella* sp.

compound **3c** (chloro group at *para* position of benzene ring) and **4c** (methoxy group at *ortho* position of benzene ring recorded lowest minimum inhibitory concentration (MIC) value of 130 µg/mL and 150 µg/mL respectively as compared to compound **1c** (no substitution on benzene ring).

## Conclusion

N-acetylated and N-propanoylated pyrazolines were synthesized by reacting different pyrazolines with acetic acid and propanoic acid in the presence of triethylamine. N-substituted pyrazolines exhibited less activity than standard ampicillin at all the tested concentrations. All the compounds showed significant differences among themselves at all the concentrations. Compounds bearing substitution of methoxy group at *meta* position and hydroxy group at *para* position of benzene ring of all N-acetylated (**7b**) and N-propanoylated (**7c**) pyrazolines were found effective against *Bacillus* sp., *Acinetobactor* sp. and *Pseudomonas* sp. but not against *Klebsiella* sp.

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## REFERENCES

1. B. Insuasty, A. Tigreros, F. Orozco, J. Quiroga, R. Abonía, M. Nogueras, A. Sanchez and J. Cobo, *Bioorg. Med. Chem.*, **18**, 4965 (2010).
2. A.E. Rashad, M.I. Hegab, R.E. Abdel-Megeid, J.A. Micky and F.M.E. Abdel-Megeid, *Bioorg. Med. Chem.*, **16**, 7102 (2008).
3. R.V. Ragavan, V. Vijayakumar and N.S. Kumari, *Eur. J. Med. Chem.*, **45**, 1173 (2010).
4. D. Kim, Y. Lee, Y.W. Lee, P.M. Dewang, Y.Y. Sheen, Y.W. Kim, H. Park, J. Yoo, H.S. Lee and Y. Kim, *Bioorg. Med. Chem.*, **18**, 4459 (2010).
5. J. Gaba, S. Sharma, G. Arora, S. Joshi and A. Goyal, *J. Indian Chem. Soc.*, **92**, 1587 (2015).
6. S. Kaur, S. Sharma, J. Kaur and P. Sharma, *Indian J. Chem.*, **52B**, 1513 (2013).
7. V.M. Barot and S.N. Panchal, *Asian J. Biochem. Pharm. Res.*, **4**, 212 (2012).